NATIONAL HEALTH LABORATORY SERVICE

MEMO

National Priority Programs, Parktown and
Centre for Tuberculosis, NICD, Sandringham

To: NHLS TB-NAAT testing laboratories, clients, and healthcare workers.

Date: 20 March 2025

Re: Implementation of stool as an additional specimen type suitable for testing by Xpert® MTB/RIF Ultra assay for the diagnosis of tuberculosis in those less than 10 years of age, as from 1 April 2025.

1. Background

Globally, approximately 1.25million children and adolescents (under 15 years) fell ill with tuberculosis (TB) in 2022 with 214'000 losing their lives from this preventable and curable disease[1]. Among deaths in HIV-negative children and young adolescents, 76% were in children less than 5 years of age with 31'000 deaths reported in those living with HIV[1]. In the same year, more than half of children and young adolescents with TB disease did not have access to life-saving TB diagnostic and treatment services[1].

Diagnosing TB in children can be challenging due to various factors, including the non-specific nature of TB symptoms (like other childhood illnesses), most young children having paucibacillary disease (i.e., harbouring relatively few TB bacilli), and difficulties in collecting specimens for diagnostic testing. Diagnosis of TB disease is usually made based on thorough clinical assessment, supported by relevant investigations and tests. Young children cannot easily produce sputum specimens and thus the use of non-sputum specimen types, that are collected in a less invasive manner, are important for diagnostic confirmation.

Every effort should be made to establish diagnostic confirmation. The World Health Organization (WHO) recommends several specimen types for the diagnosis of pulmonary TB in children and adolescents, the choice depending on how young the child is and the feasibility and acceptability of the collection. Non-invasively collected specimens include expectorated sputum, induced sputum, and stool. Invasively collected specimen includes gastric and nasopharyngeal aspirates[2].

An important new development is the recommendation for the use of stool as a non-invasively collected specimen for diagnostic confirmation of respiratory TB in children[2]. Children with TB-disease tend to swallow their sputum secretions (containing TB bacilli). The swallowed TB bacilli pass through the digestive tract and thus can be detected in stool specimens by the Xpert® MTB/RIF Ultra assay. Stool is thus regarded as a 'respiratory' specimen to confirm TB.

In a rapid communication, published 24 September 2024, WHO updated its recommendations for the diagnosis of TB and detection of drug-resistance in children[3]:

- *In children who have signs or symptoms or screened positive for pulmonary TB, concurrent testing using low-complexity automated nucleic acid amplifications tests (NAATs) on respiratory specimens and stool should be used as the initial diagnostic strategy for diagnosing TB rather than low-complexity automated NAATs on respiratory or stool samples **alone**.*
- *The products for which eligible data met the class-based performance criteria for low-complexity NAATs were Xpert® MTB/RIF Ultra (Cepheid, Sunnyvale, USA).*

[1] https://cdn.who.int/media/docs/default-source/hq-tuberculosis/infomation-sheet_roadmap-towards-ending-tb-in-children-and-adolescents-3rd-ed.pdf?sfvrsn=ed5aaddb_3&download=true. Accessed 04 March 2025.
[2] https://iris.who.int/bitstream/handle/10665/352523/9789240046832-eng.pdf?sequence=1. Accessed 04 March 2025.
[3] https://www.who.int/publications/i/item/B09111. Accessed 04 March 2025.

2. The information in this section is aimed at healthcare workers:

2.1 Procedure for collecting stool specimens from <10-year-olds for Xpert® MTB/RIF Ultra testing

- As per the recommendations, stool specimens will be collected in addition to other specimen types (such as sputum or gastric washings), should pulmonary TB be suspected.
- *Addendum 1* provides guidance for healthcare workers on '*How a stool specimen should be collected from a child?*' including a list of frequently asked questions.

3. The information in this section is aimed at NHLS laboratory staff:

3.1 Laboratory footprint for implementation of stool as an additional specimen for testing by Xpert® MTB/RIF Ultra

Aligning to the updated WHO recommendations, the NHLS is introducing stool, as an additional specimen type in <10-year-olds, for processing by Xpert® MTB/RIF Ultra assay. Eighteen (18) NHLS laboratories have been earmarked for Xpert® MTB/RIF Ultra stool testing, listed by region in Table 1.

Table 1: Centralised NHLS laboratories earmarked for processing stool specimens by NHLS region.

Region	**NHLS laboratory testing stool by Xpert® MTB/RIF Ultra in <10years, as from 1st of April 2025**
Eastern Cape	• Port Elizabeth • Nelson Mandela
Free State and North West	• Universitas • Tshepong
Gauteng	• Braamfontein • Tshwane Academic • Dr George Mukhari • Chris Hani Baragwanath Hospital • Helen Joseph • Charlotte Maxeke Johannesburg Academic Hospital
KwaZulu-Natal	• Inkosi Albert Luthuli Central Hospital • King Edward VIII
Limpopo and Mpumalanga	• Polokwane • Ermelo
Western and Northern Cape	• Greenpoint • Tygerberg • Groote Schuur • Kimberley

3.2 Procedure for receiving stool specimens at the NHLS

- When a stool specimen has been received stipulating either of the following requests: TB-testing; GeneXpert; GeneXpert MTB/RIF; GeneXpert Ultra; GXP; GXPU; or TB-NAAT, the following test and test code should be requested on the LIS:
  - Specimen type: select **stool**.
  - Test code: select **M326**.
  - Test name: select **GeneXpert Ultra**.

- The age of the patient should be captured on the LIS. **The request for stool testing is only valid where age <10 years**.
- Stool specimens for Xpert® MTB/RIF Ultra testing should be rejected where the age ≥10 years or has not been specified.
- Figure 1 provides an example of a typical request where the specimen type has been indicated as stool and the GeneXpert test requested.

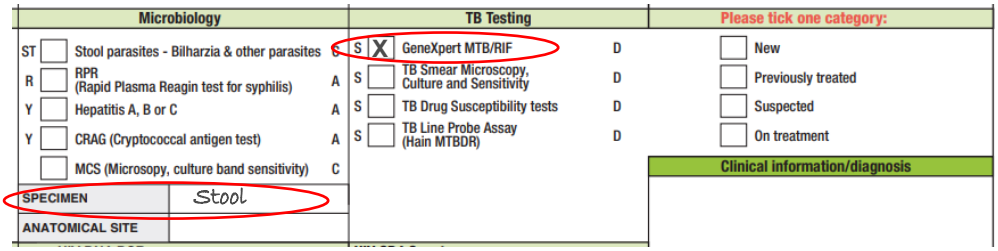

Figure 1: An example of how a stool specimen for Xpert® MTB/RIF Ultra may be requested.

- Stool specimens may be received alone or accompanied by other specimen types (such as sputum or gastric aspirates, etc.) for TB-testing. **Only the stool specimen should be referred to one of the 18 designated Xpert® MTB/RIF Ultra stool testing sites (listed in Table 1)**. The other specimen types (such as sputum or gastric aspirates, etc.) should follow the routine workflow at the respective testing site(s).
- The date from which NHLS will commence receipt and processing of stool specimens**: 1st of April 2025.**

3.3 Testing of stool by Xpert® MTB/RIF Ultra at the designated NHLS laboratories

- The Xpert® MTB/RIF Ultra testing procedure for stool differs from other specimen types. A pre-processing step is required and is defined as the Simple-One-Step (SOS) method[4,5] . It is applicable only for use on the Xpert® MTB/RIF Ultra assay. No data is yet available for using stool and the SOS method on the BD MAX MDR-TB or the Roche cobas MTB/RIF-INH assays.
- The initial processing[6] is based on whether the collected stool is predominantly 'solid' or 'liquid' in nature, as determined by the Bristol stool classification chart[7].
- The SOS method does not involve centrifugation and relies on natural sedimentation (by gravity) to allow particulate matter to settle, with only the supernatant being removed for insertion into the testing cartridge.
- Standard operating procedures (SOP) guiding the laboratory processing of stool specimens are available for access via Q-Pulse (national SOP number: GPL4541).

3.4 Reporting of Xpert® MTB/RIF Ultra results for stool testing by NHLS laboratories

- The interface reporting rules for Xpert® MTB/RIF Ultra results for stool testing are identical to that in place for any other specimen type tested on the same assay.

[4] de Haas P, Yenew B, Mengesha E, Slyzkyi A, Gashu Z, Lounnas M, Tesfaye E, Bedru A, Tiemersma E, Kremer K, mare M, Diriba G, Zerihun B, Gudina T, Tegegn B, Bonnet M, Negeri C, Klinkenberg E. 2021. The simple one step (SOS) stool processing method for use with the Xpert MTB/RIF assay for a childfriendly diagnosis of tuberculosis closer to the point of care. J Clin Microbiol 59:e00406-21. https://doi.org/10.1128/JCM.00406-21.
[5] Yenew B, de Haas P, Diriba G, Kebede A, Sherefdin B, Demissie Y, Bedru A, Sahile M, Mengesha E, Dememew ZG, Tegegn B, Slyzkyi A, Amare M, Getahun M, Abdella S, Jerene D, Tiemersma E. Optimization of the Simple One-Step Stool Processing Method to Diagnose Tuberculosis: Evaluation of Robustness and Stool Transport Conditions for Global Implementation. Microbiol Spectr. 2023 Aug 17;11(4):e0117123. doi: 10.1128/spectrum.01171-23. Epub 2023 Jun 26. PMID: 37358407; PMCID: PMC10434014.
[6] https://www.kncvtbc.org/uploaded/2021/03/SOS-Stool-Method-poster-A51.pdf. Accessed 06 March 2025.
[7] https://www.kncvtbc.org/uploaded/2021/03/Stoolbox-SOP1.pdf. Accessed 06 March 2025.

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Acknowledgements:

- *Dr Juli Switala*, Specialist Paediatrician, for compilation of the 'clinical guidance on specimen collection'.

Compiled by:

- National Priority Programmes, National Health Laboratory Service:
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ADDENDUM 1

CLINICAL GUIDANCE ON SPECIMEN COLLECTION

How should a stool specimen be collected from a child?

- This will vary according to age and setting.
- The first bowel movement of the day is ideal.
- A few possibilities to consider:
    1. A child who still uses diapers:
        - Consider turning the diaper inside out, to minimise absorption of the stool into the diaper material, prior collection.
        - A specimen can be taken directly from the diaper.
        - Ideally, minimise the contact time between the stool specimen and the diaper as substances within the diaper may affect test results.
    2. A child who no longer needs to be in diapers:
        - The child may still be able to wear and defecate into a diaper for the purpose of specimen collection.
    3. A child that can use a 'potty' or bedpan:
        - In this setting, the child should pass urine first, since mixing of urine and stool could affect the results.
        - Following urination, a large piece of toilet paper or a linen protecter can be placed on the floor or into the potty/bedpan for the child to pass stool onto. Once the specimen is collected from the toilet paper/linen protector, the same can be appropriately disposed of.
        - The use of paper/linen protector provides a barrier between the stool specimen and residual cleaning products or disinfectant substances (used to clean the potty/bedpan which can affect results) and from contamination from previous users.
    4. A child that can use a toilet:
        - The child may be prepared to use a potty/bedpan as described above.
        - An alternative collection option is to place a linen saver or sheet of plastic ('gladwrap') between the toilet bowl and the seat, loosely enough to create a catchment 'sag' to 'catch' stool without spillage, but tight enough such that it does not to collapse into the toilet bowl.
        - Once the specimen is taken into the specimen container, the remaining stool can be flushed into the toilet and the linen saver/plastic discarded appropriately.
        - A child old enough to pass stool into a toilet independently may also be able to produce a sputum specimen with encouragement or with/without induction.

Additional notes on collection of stool specimens from <10-year-olds for Xpert® MTB/RIF Ultra testing

- Stool with a more solid consistency is preferred than very liquid specimens, however this may not always be feasible, and the laboratory can process both stool types (solid and liquid consistency).
- Once the stool specimen has been collected it should be placed into a universal specimen container with the standard screw-on lid. Use of containers with screw-on lids will minimise leaks. Containers with snap-on lids should not be utilised. Figure 2 differentiates universal specimen containers with 'screw-top' from those with 'snap-on' lids.
- Containers should be clean and dry prior to adding the stool specimen. The presence of water, urine, or disinfectants can affect the result.

- The container should not be filled to more than half its capacity to minimise leakage. The container can be wrapped in tissue or other absorbent material and placed in routine plastic laboratory packet, utilised for other respiratory specimens, and sealed.
- Request forms should be clearly labelled with patient's identifiers (including age), hospital number, date and time of collection. The request form must specify stool as the specimen type and GeneXpert should be the test requested. Other tests should not be requested on the same stool specimen.
- Should patient age not be included in the specimen request form, the specimen will be rejected.
- Using the routine NHLS courier service, the specimen should be transported to the laboratory as soon as feasibly possible. Provided stool specimens are stored out of direct sunlight, they can be stored for 48 hours at room temperature. Once received at the assigned testing laboratory, specimen preparation and testing should commence as soon as possible.

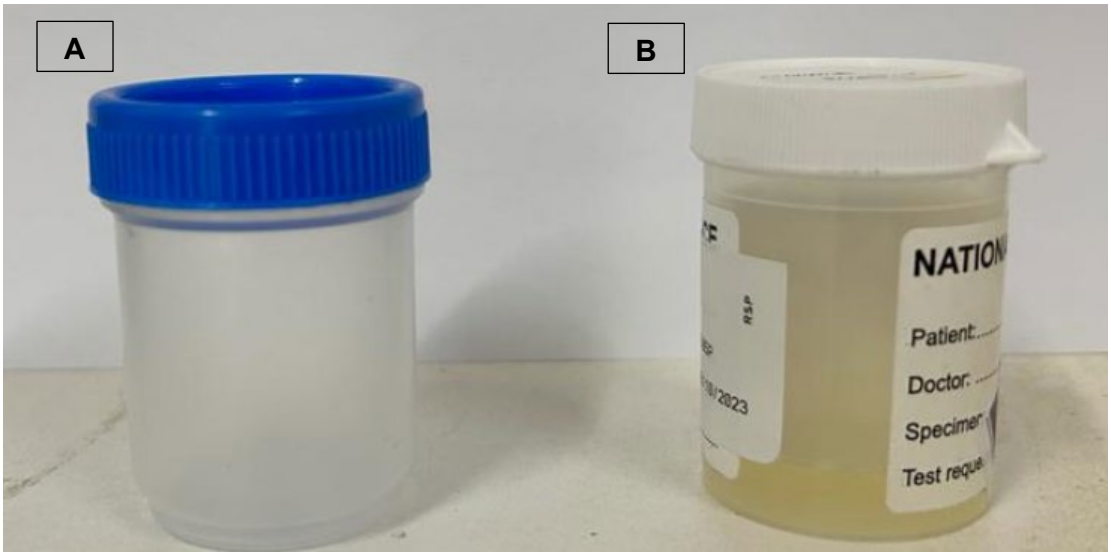

Figure 2: A: Example of a universal specimen container with a screw-on lid. This is the recommended specimen receptacle for stool collection. Note that the colour of the screw-on lid may vary from blue, green, or white. Refrain from using universal specimen containers with snap-on lids for stool specimen collection (as detailed in B).

Frequently asked questions: stool as an additional specimen type for diagnosis of paediatric TB-disease

1. *What is the best specimen type for tuberculosis disease detection in children?*

- A spontaneous or induced sputum, followed by a gastric aspirate are most likely to yield positive laboratory results in children with tuberculosis.
- It is important to remember that no specimen types perform as well in children, compared to adults, as children tend to have paucibacillary disease.
- A negative TB test from the laboratory does not exclude TB, and should be viewed in context of history, clinical examination and other investigations, e.g., chest X-ray, etc.

2. *Why should we take specimens from children if negative results cannot exclude tuberculosis?*

- A positive result may provide the evidence needed to justify treatment if there is not sufficient clinical evidence.
- A laboratory result is the only way to detect drug susceptibility which affects regimen selection.
- A negative result in a child contact can add to confidence in initiating tuberculosis preventive treatment.

3. *Why are we considering stool as a specimen for pulmonary tuberculosis for children under 10 years of age?*

- Material from the lungs or airways may move up the airway to the back of the throat during talking, coughing or breathing, in children.
- This material then gets swallowed into the stomach and passes through the gastrointestinal tract and is then processed as stool.
- If there is active tuberculosis disease in the lungs, some organism genetic material may be detected by the assay in stool specimens.

4. *What are the advantages of collecting and testing of stool specimens for detection of tuberculosis disease?*

- Due to challenges collecting pulmonary specimens from children, limited access to chest X-rays at community level, and referral to higher levels of care often resulting in diagnostic delays or disengagement from care, stool specimen collection provides an option that will be more feasible for some children.

5. *What are the disadvantages of collecting and testing of stool specimens for detection of tuberculosis disease?*

- Stool specimens (compared to other specimen types) are less likely to yield positive nucleic-acid amplification test results. However, as per recommendations from the World Health Organisation, stool could be collected in addition to sputum specimens to improve diagnostic yield.
- Stool specimens are only suitable for Xpert® MTB/RIF Ultra testing and are not validated on other testing platforms.
- Stool is not suitable for tuberculosis culture due to the high faecal bacterial counts.

End